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PKCβ (D3E7O) Rabbit mAb

#46809

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Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, F Endogenous	H, M, R	80 kDa	Rabbit IgG**

Background: Activation of protein kinase C (PKC) is one of the earliest events in a cascade that controls a variety of cellular responses, including secretion, gene expression, proliferation, and muscle contraction (1,2). PKC isoforms belong to three groups based on calcium dependency and activators. Classical PKCs are calcium-dependent via their C2 domains and are activated by phosphatidylserine (PS), diacylglycerol (DAG), and phorbol esters (TPA, PMA) through their cysteine-rich C1 domains. Both novel and atypical PKCs are calcium-independent, but only novel PKCs are activated by PS, DAG, and phorbol esters (3-5). Members of these three PKC groups contain a pseudo-substrate or autoinhibitory domain that binds to substrate-binding sites in the catalytic domain to prevent activation in the absence of cofactors or activators. Control of PKC activity is regulated through three distinct phosphorylation events. Phosphorylation occurs *in vivo* at Thr500 in the activation loop, at Thr641 through autophosphorylation, and at the carboxy-terminal hydrophobic site Ser660 (2). Atypical PKC isoforms lack hydrophobic region phosphorylation, which correlates with the presence of glutamic acid rather than the serine or threonine residues found in more typical PKC isoforms. The enzyme PDK1 or a close relative is responsible for PKC activation. A recent addition to the PKC superfamily is PKCμ (PKD), which is regulated by DAG and TPA through its C1 domain. PKD is distinguished by the presence of a PH domain and by its unique substrate recognition and Golgi localization (6). PKC-related kinases (PRK) lack the C1 domain and do not respond to DAG or phorbol esters. Phosphatidylinositol lipids activate PRKs, and small Rho-family GTPases bind to the homology region 1 (HR1) to regulate PRK kinase activity (7).

PKCβ has two isoforms, PKCβI and PKCβII, due to alternative splicing (8).

Specificity/Sensitivity: PKCβ (D3E7O) Rabbit mAb recognizes endogenous levels of total PKCβ protein. The antibody recognizes both PKCβI and PKCβII isoforms.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Pro619 of human PKCβI protein.

Background References:

(1) Nishizuka, Y. (1984) *Nature* 308, 693-8.

(2) Keranen, L.M. et al. (1995) *Curr Biol* 5, 1394-403.

(3) Mellor, H. and Parker, P.J. (1998) *Biochem J* 332 (Pt 2), 281-92.

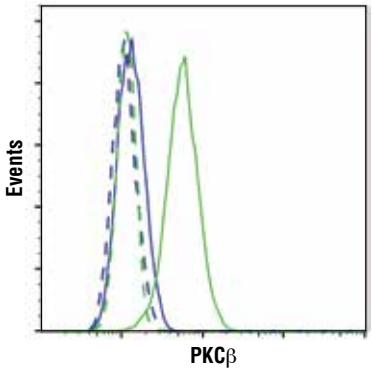
(4) Ron, D. and Kazanietz, M.G. (1999) *FASEB J* 13, 1658-76.

(5) Moscat, J. and Diaz-Meco, M.T. (2000) *EMBO Rep* 1, 399-403.

(6) Baron, C.L. and Malhotra, V. (2002) *Science* 295, 325-8.

(7) Flynn, P. et al. (2000) *J Biol Chem* 275, 11064-70.

(8) Kawakami, T. et al. (2002) *J Biochem* 132, 677-82.



Flow cytometric analysis of HeLa cells (blue) and K-562 cells (green) using PKCβ (D3E7O) Rabbit mAb (solid lines) or a concentration matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (dashed lines). Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.*

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:50
Flow Cytometry	1:200

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

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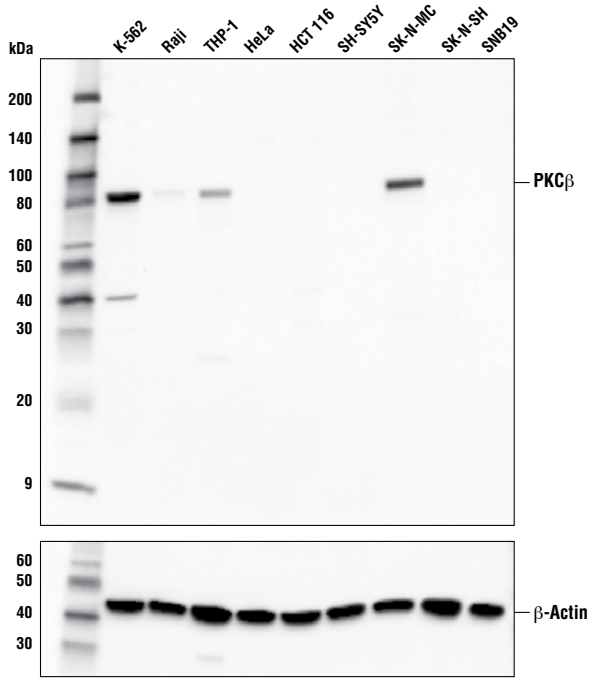
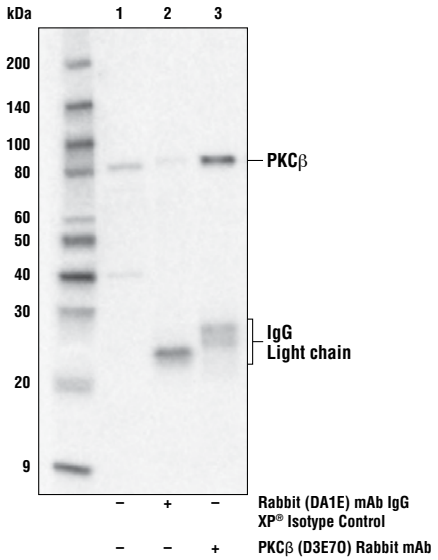
Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Western blot analysis of extracts from various cell lines using PKCβ (D3E7O) Rabbit mAb (upper) or β-Actin (D6A8) Rabbit mAb #8457 (lower).



Immunoprecipitation of PKCβ from K-562 cell extracts. Lane 1 is 10% input, lane 2 is Rabbit (DA1E) mAb IgG XP® Isotype Control #3900, and lane 3 is PKCβ (D3E7O) Rabbit mAb. Mouse Anti-rabbit IgG (Light-Chain Specific) (D4W3E) mAb (HRP Conjugate) #93702 was used for detection.